Structure
Previews

Key Determinants of Rab Specificity

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Structures are reported for complexes between Rab27 and the Rab27-binding domains of two molecules involved in successive steps in melanosome transport. Comparison of the Rab27B:Slac2-a (Chavas et al., 2008) and Rab27A:Slp2-a (Kukimoto-Niino et al., 2008) structures with that of the Rab3A:Rabphilin complex gives important clues to the specificity of the interactions.

Rab proteins are essential regulators of the important process of intracellular vesicular transport (Zerial and McBride, 2001). Paramount among the multiple tasks they fulfill is the physical connection of transport vesicles to motor systems to allow the actual movement from a donor to an acceptor membrane, and, once there, the specific recognition of a membrane or membrane domain before fusion of the vesicles and the membranes occurs. One of the over 60 members of the human Rab family, Rab27 has attracted much attention in recent years, partly because of its role in several diseases, including Griscelli syndrome (Seabra et al., 2002). There are two closely related Rab27 homologs, Rab27A and Rab27B, which appear to play similar but not identical roles, with cell-type specific expression of Rab27B being more restricted. Rab27 is attached by two C-terminal geranylgeranyl residues to secretory granules in many secretory cells and to pigment-containing melanosomes in melanocytes. The final stage of transport of melanosomes to the dendritic regions of the cell occurs on actin filaments. This happens via Rab27A interaction with Slac2-a (melanophilin), which in turn binds to the motor-protein myosin Va. In Griscelli syndrome (GS), there are genetic defects in myosin Va (GS1), Rab27 (GS2) or melanophilin (GS3). All forms are characterized by hypopigmentation, with additional severe immunodeficiency and neurological defects in GS2. Furthermore, Rab27A plays a role in the process of docking to the plasma membrane. The second Rab27 effector protein, Slp2-a (exophilin 4), interacts via its effector domain with Rab27 and via its 2 C-terminal C2 domains to the lipid phosphatidylserine in the plasma membrane. Thus, two different Rab27 effector proteins are involved in two successive steps of the transport mechanism. In this issue of *Structure*, structures of the Rab27-binding regions of Slac2-a and Slp2-a bound to Rab27 are presented.

Slac2-a and Slp2-a both have their Rab27-binding domains (referred to as SHD [for Slp-homology domains], or more specifically as RBD27 domains) near or at their N terminus. These consist of two α-helical regions linked either directly (Slp2-a) or via a zinc-binding subdomain (Slac2-a) of unknown function (FYVE-related domain). As shown in Figure 1, the overall structures of the two complexes are similar, and there is an obvious similarity to the previously determined structure of the Rab3A:Rabphilin complex (Ostermeier and Brunger, 1999), which, like Slac2-a, has a zinc-binding domain. Unlike Slac2-a, Slp2-a, and a number of other identified Rab27 effectors that are highly specific, Rabphilin belongs to a group of effectors that interact not only with Rab27 but also with other exocytic Rabs, in particular Rab3 and Rab8. It is therefore of interest to compare the structures of the three different complexes to understand the origins of the specificity.

In all three complexes, the main regions of the Rab proteins involved in the interactions are the CDRs (complementarity determining regions [Ostermeier and Brunger, 1999]) and the switch regions (1 and II) that have GTP/GDP sensitive conformations. Of the large number of interactions that can be identified, some of the most important (i.e., involving residues conserved between all three complexes or that play a decisive role in specificity) are shown in the lower part of Figure 1. In the report on the Rab27B:Slac2-a structure, four Rab residues were identified as key determinants for the specific interaction of Slac2-a with Rab27B rather than Rabphilin. These residues are Y6 from the N-terminal CDR, and from switch II L84, F88, and D91. In keeping with this, replacing these residues by their counterparts in Rab3A led to reduction or complete loss of binding of Rab27B to Slac2-a. Thus, the Y6F and D91G mutants are reported to show severely reduced Slac2-a binding, and the quadruple mutant showed no detectable interaction. Conversely, replacement of the four corresponding residues in Rab3A by their Rab27B counterparts led to significant Slac2-a binding activity. In the report on the Rab27A:Slp2-a structure, it is suggested that Y122 of Rab27 (a single amino acid insertion in comparison with Rab3A) plays a crucial role in the specific interaction with Slp2-a. However, a Rab27 mutant with this residue mutated to cysteine still interacts with Slac2-a (Fukuda, 2002) and it does not appear to induce affinity for Slac2-a when introduced into Rab3A.

The results reported in the two contributions to this issue of *Structure* significantly extend our understanding of the specificity of Rab-effector interactions. They show that the differential interactions are not caused by the principal changes of effector structure but by the changes in individual amino acids or small groups of amino acids. Even here, the effects at the structural level can be relatively subtle. Thus, substitution of the key residue Y6 in Rab27B by its Rab3A phenylalanine counterpart would not be expected to destroy the hydrophobic interaction with R35 in Slac2-a or R32 in Slp2-a, since the Rab3A:Rabphilin shows a similar interaction (with R71 in Rabphilin). However, the additional -OH group on Y6 allows a hydrogen bonding interaction to E32 in Slac2-a and to E29 in Slp2-a. One of the other key residues, D91 in Rab27A/B, allows further interactions in addition to the

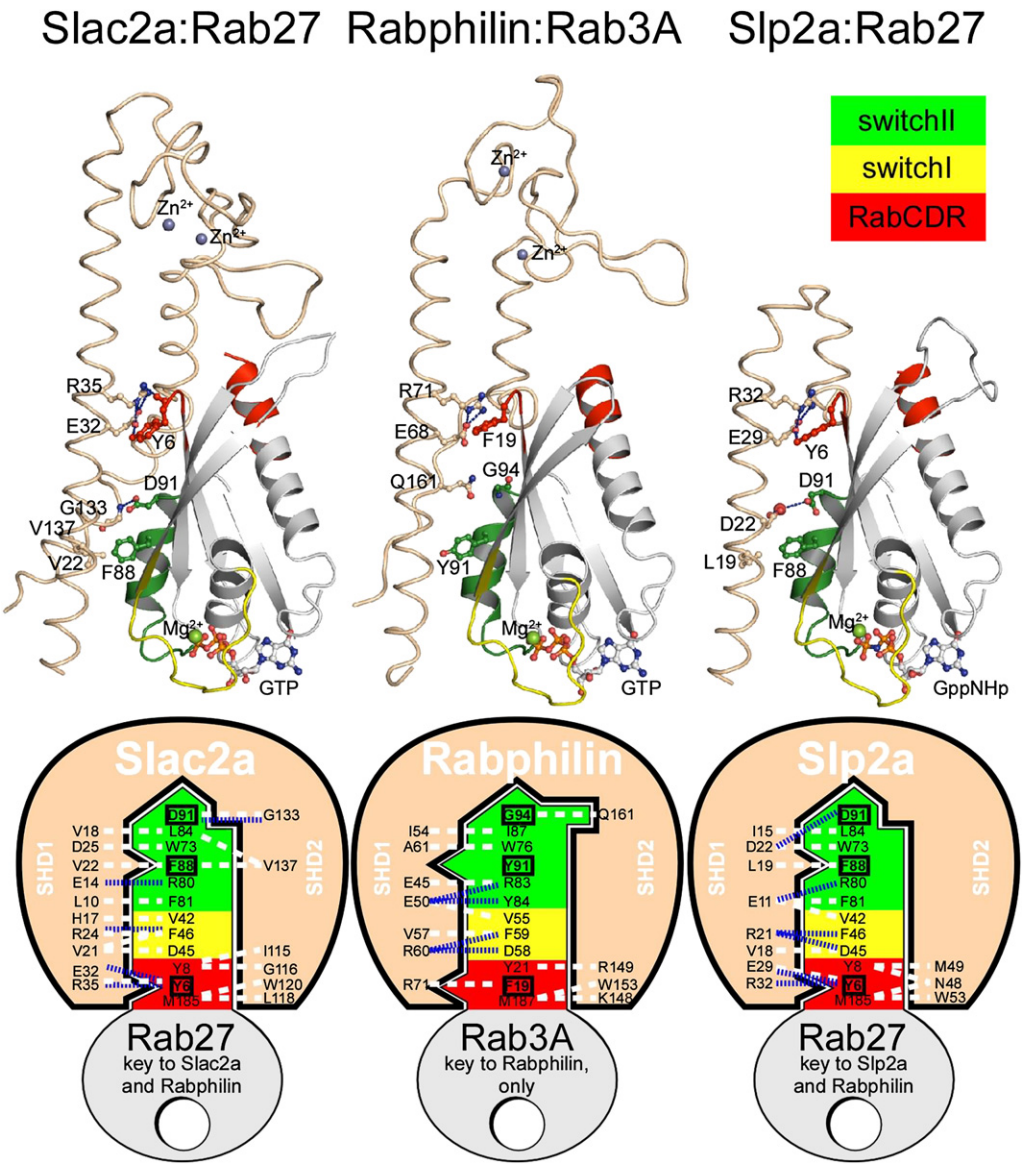

Figure 1. A Lock-and-Key Model of Rab-Effector Binding Specificity

The figure highlights structural features of the Rab27B:Slac2-a (left column), Rab3A:Rabphilin (middle column), and Rab27B:Slp2-a complexes (right column). (Top panel) Key residues Y6(Rab27)/F19(Rab3A), F88(Rab27)/Y91(Rab3A), and D91(Rab27)/G94(Rab3A) are indicated as a ball-and-stick model in the three Rab:effector-complexes together with their interacting partners. The Rab-proteins are shown as cartoon models; the effectors are drawn in ribbon representation. (Bottom panel) Schematic overview of conserved amino acid interactions between Rab and the effector molecules. This representation emphasizes the role of the key residues (boxed amino acids) as determinants for the interaction between Rab27B:Slac2-a, Rab3A:Rabphilin, and Rab27A:Slp2-a. The presence of Y6, F88, and D91 permits Rab27 to bind to Slac2-a, Slp2-a, and Rabphilin. In contrast, the residues F19, Y91, and G94 allow Rab3A to bind to Rabphilin only. Blue dashes: polar interactions; white dashes: hydrophobic interactions; SHD: Slp homology domains 1 and 2; red sphere: water molecule.

van der Waals contacts seen in the case of G94 in Rab3A. However, these interactions are different in the Slac2-a and Slp2-a complexes, with a water-mediated side chain interaction in the former and a side chain-backbone interaction in the latter complex. In the contribution describing the Rab27A:Slp2-a complex, it is suggested that a shift in the orientation of the conserved Rab-binding motif (S/T)(G/L) x $W(F/W)_2$ in Slp2-a compared with the orientation in the Rab3A:Rabphilin structure also contributes to specificity.

The different specificities of the Rab27 effectors discussed here appear to arise as the sum of a number of relatively small structural effects. Their detailed understanding is hampered at several levels, one of which is connected with making predictions about interaction-free energies based on determined structures. Thus, it has been reported that Rab27A binds to Slp2-a with ca. ten-fold higher affinity than to Slac2-a (Fukuda, 2006). However, there is no obvious explanation for this on the basis of the structures presented. An additional problem is the fact that there are not enough data on the affinities and kinetics of Rab-effector interactions, and on the effects of mutations on these. Thus, most of the statements made here and in related publications are based on pull-down experiments,

which are at best qualitative and at worst misleading. The more quantitative data mentioned above were obtained by surface plasmon resonance (SPR) and have been used as the basis for an initially attractive model to explain the manner in which Rab27 on the surface of melanosomes is passed on from its interaction with Slac2-a to allow transport, to its interaction with Slp2-a to allow docking onto the plasma membrane (Fukuda, 2006). However, there are many possible additional factors that could make a simple comparison of affinities inadequate. For example, the relative concentrations, or local densities, of the effectors could play a dominating effect. Another possibility is that the FYVE-related domain of Slac2-a is, like the classical FYVE-domains, a lipid-binding moiety and that an interaction with the melanosome membrane increases the effective affinity of Rab27 to Slac2-a. As in many areas of biology, real numbers are needed here for affinities and rate constants, as well as for effective concentrations of interaction partners, before the system can be understood quantitatively. The structures presented here are an excellent starting-off point for such an analysis.

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Tripping a Switch: PDZRhoGEF rgRGS-Bound Gα13

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Ras superfamily guanine nucleotide-binding proteins, such as G-proteins and small GTPases, are a paradigm for two-state molecular switches in cell signaling. Recent experimental and theoretical studies question this simple model. Now Chen et al. (2008) provide evidence that the rgRGS domain of PDZRhoGEF has evolved to "trip" the nucleotide-dependent switch of the subunit of the heterotrimeric G-protein, Gα13, so that it can also bind to its GDP state.

The ras superfamily of proteins provides a common α/β fold to bind guanine nucleotides, either GTP or GDP. The terminal phosphate group difference in the nucleotides is accommodated by structural and dynamic adjustments of several regions of the fold, principally in so-called switch regions. Regulatory proteins can sense these conformational differences and bind to G-proteins and small GTPases in a GDP- or GTP-selective manner. Essentially, without exception, the proteins are active for signaling in the GTP-bound state and are unable to bind effector proteins, or bind them very weakly, in the GDP state. This feature has made G-proteins and small GTPases a paradigm for two-state signaling. Both small GTPases and G-proteins posses a weak intrinsic GTPase activity, hydrolyzing GTP to turn off their signaling function. Regulatory proteins, GTPase-activating proteins (GAPs), are typically needed to accelerate the GTP hydrolysis and guanine exchange-factor proteins (GEFs) are needed to eliminate the GDP and reload the G-protein/small GTPase with GTP. Several decades of extensive structural biology and protein biophysics investigations have played a critical role in understanding the function of these proteins (Oldham and Hamm, 2006); the report by Chen et al. (2008) in this issue illustrates the level of detail and insight that is provided by a protein crystallography study of an unusual system, challenging our conventional understanding of G-protein function. However, the (at first sight) odd-ball behavior is well in line with what is shaping up to become a shift in the G-protein/GTPase signaling paradigm.

Sprang and colleagues have been studying the rgRGS family of multidomain/multifunctional proteins, which are effectors of G-protein Gα13 and become active as GEFs for the small GTPase RhoA (Sternweis et al., 2007). Binding to Gα13 occurs via two regions, a well-conserved rgRGS domain and an acidic region/IIG motif, at the N terminus of this domain. Even though the surface regions